FIGURE 1A

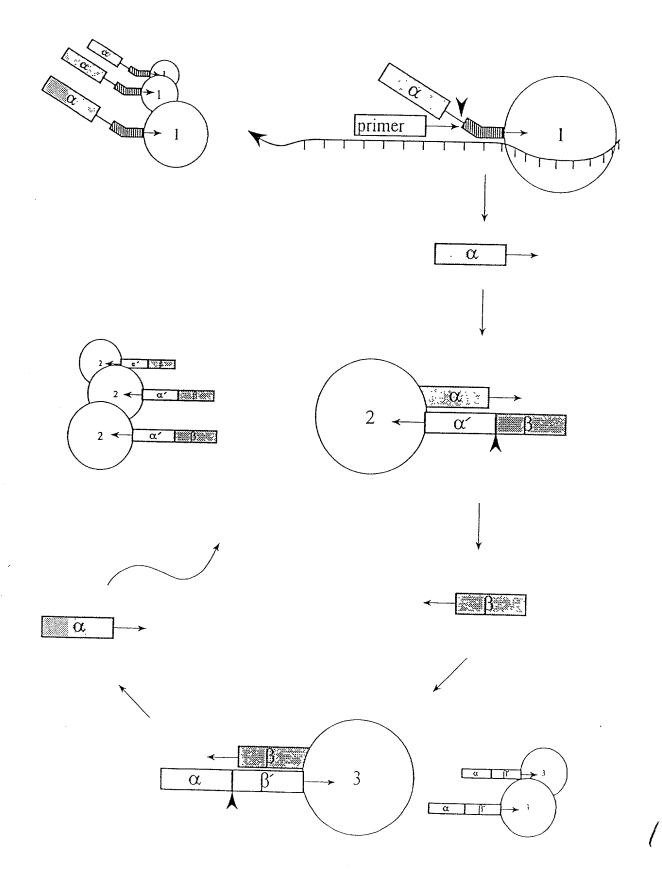
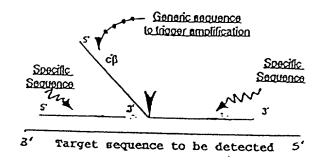
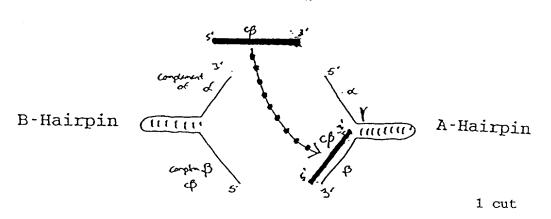


FIGURE 1B

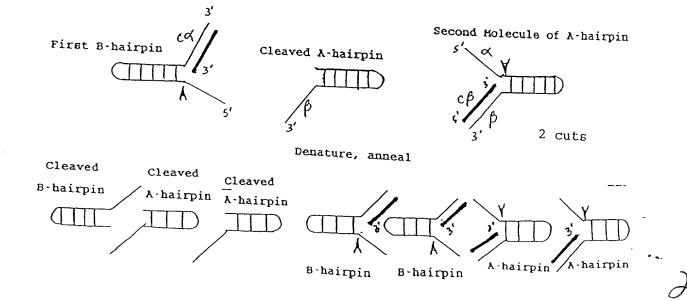
PART ONE: TRIGGER REACTION



PART TWO: DETECTION REACTION



Denature, anneal



70	2	140 137 140		207 204 210		277 274 280		347 344 350
0000001110	GCACCTTCTTCGCCCTGAAGGGC		C G C C A A G A G C C C T C A A G G C C C T G A A G G A G G A G G G G G C X X G G C G G T G X T C G T C T T T G A C G C C A A G	A	G C C C C C C C T T C C G C C C A C G C C T A C G G C C C A G G C C C C C C C C C C		CCCGGCAGCT CGCCCT CAT CAAGGAGCT GGT GGACCT CCT GGGGCT T GCGCGCCT CGAGGT CCCCGGCTA	6 6
MAJORITY (SEQ ID NO:7) DHAPTAD (SEQ ID NO:1) CHAPTR (SEQ ID NO:2) PARPTR (SEQ ID NO:3)								
MAJORITY DNAPTAD CNAPTR		ONAPTAO CNAPTH. CNAPTH	MAJORITY	ONAPTAO CNAPTR CNAPTTH	MAJORITY	ONAPTAO CNAPTR CNAPTTH	MAJORITY	ONAPTAO CNAPTE CNAPTTH

The state of the s

	417 414 420		487 484 490		557 554 560		627 624 630		694 691 700
C G A G G G G G G G G G G T G G C C G C C G C C C G G A A G G C G G A A A G G A G G G G	T	A CC G C C G A C C C C C C C C C C C C C	TAAAT	T CA C C C C C C C C C T T T C C C A C A	G. G. C.	G G G G G G C C C C C C C C C C C C C C	66A6TABGA6A	GAGT GGG GGA G C CT G GAAA G C T C GAA G C T G GA C C G G G T GAA G C C C C C C · · · C X T C C G G G A G A G A	
MAJORITY (SEQ ID NO:7)	(SEQ ID NO:1) (SEQ ID NO:2) (SEQ ID NO:3)								
MAJORITY	DNAPTAD CNAPTR. CNAPTTH	MAJORITY	ONAPTAD CNAPTH. CNAPTH	MAJORITY	ONAPTAO CNAPTE. CNAPTIH	MAJORITY	ONAPTAD CNAPTR CNAPTTH	MAJORITY	ONAPTAO CNAPTEL CNAPTTH

	764 761 770		834 831 840		904 901 910		974 971 980		1044 1041 1050
T CCA G G C C C C A C C A X G A X G C T G T C T C C T G G G A G C T X T C C C A G G T G C C A C C T G C C C C C C C C A C C A C C T G C C C C C C C C C C A C C T C C C C C C	T	G G T G G A C T T C G C C C A A G X G G G G G G C C C G G G C G G G G C C T T A G G G C C T T T C T G G A G G C T G G A G T T T	A	G G C A G C C I C C A C G A G T T C G G C C C T C G A G G G C C C C A A G G C C C T G G A G G C C C C C C C C C C C C C C C		C G G A A G G G G C C T T C G G C C T T T G C C C C		C G C C C C C C G G G G G G G G G G G G	T. 66; . 6T
MAJORITY (SEQ ID NO:7)	(SEQ ID NO:1) (SEQ ID NO:2) (SEQ ID NO:3)								
MAJORITY	DNAPTAD CNAPTR CNAPTH	MAJORITY	ONAPTAD CNAPTR CNAPTTH	МАЛОВПТ	ONAPTAD CNAPTR. CNAPTTH	MAJORITY	ONAPTAD CRUPTE CRUPTE	MAJORITY	ONAPTAD CNAPTR CNAPTTH

-()			X		4 - 0		4 - 0
1111	1181		1254 1251 1260		1324 1321 1330		1394 1391 1400
66ACCTGGCCGTTTTGGCCCTGAGGGGCCGTXGACCTCXTGCCCGGGGGCAC AAGGGG	ACCCCAT GCT CCT ACCT CCT GGA CCCCT CCAA CACCA CCC CCG CCC CGC CCCCCCT ACCC	GGGGGAGTGGAGGGAXGCGGGGGGGGGGCCCTCCTXTCCGAGAGGCTCTTCCXGAACCTXXXGGAG	6	C G C C T T G A G G G G G G G G G G G C T T T G G C T T T A C C A G G A G G G G A G G C C C C T T T C C C G G G T C C T G G	A. G A A A	C C C A C A T G C A G G C C C G G G G G T X C G G C C T G G C C C T A C C T C C C C C C T X T C C C T G G A G G T G C C G G A	66 66
(SEQ ID NO:7) (SEQ ID NO:1) (SEQ ID NO:2) (SEQ ID NO:3)							
MAJORITY DNAPTAD CKAPTH. CNAPTTH	MAJORITY DNAPTAD CNAPTR CNAPTR	маловпт	ONAPTAD CNAPTR CNAPTTH	MAJORITY	DNAPTAD CNAPTR CNAPTTH	MAJORITY	ONAPTAD CNAPTR CNAPTR

FIGURE 2 (cont'd)

	1464 1461 1 <i>4</i> 77		1534 1531 1540		1604 1601 1610		ا اد, 1680		1744 1741 1750	
G G A G A T C C G C C C C C C C A G G A G G T C T T C C G C C T T G G C C C C C	6.6 A.6 6 T T T 6	CAGCT GGAAAGGGT GGT CTTT GA CGAGGT X GGGGTT CC CGC CAT CGGCAAGAGGGGAGAGAGACXGGCAAGG		GCT CCA CCAGCG CGC CGT GGT GGT GGC CCT X CGX GAG GC CCC CCC CAT CGT GGAGAGAT CCT GCAGTA		CCGGGAGCT CACCAAGCT CAAGACACCTACATXGACCCCCT GCCXGXCCT CGT CGACCCCAGGACGGGC		C G C C T C C A C A C C C C C T T C A A C C A C G C C C A C G C C A C G C C A G G C T T A G T A G C T C C C A A C C T G C	6	
MAJORITY (SEQ ID NO:7)	(SEQ ID NO:1) (SEQ ID NO:2) (SEQ ID NO:3)									
MAJORITY	ONAPTAD CNAPTR CNAPTR	MAJORITY	DNAPTAO DNAPTR. DNAPTTH	MAJORITY	DNAPTAO DNAPTR DNAPTTH	MAJORITY	DNAPTAD CNAPTEL CNAPTTH	אלחאטראאו	ONAPTAO CNAPTR CNAPTTH	

7

MAJORITY (SEQ ID NO:7) AGAACAT CCCCG
GTTGGTGGCCCTGGAGT
AT C C G G G T C T T C C A G G A
AGGCGGTGGACCCCC
A. GG. A T
CCACCCCTCTCCCA
TA. 6.

	MAJORITY (SEQ ID NO:7)	MAJORITY (SEQ 1D NO:7) AGGIT CCCCAAGGI GCGCGCGCGT GGATIGAGAGCCCT GGAGGGGGGGGGGGGGGGGGGGGGGGGGGG
	DNAPTAD (SEQ ID NO:1) DNAPTR (SEQ ID NO:2) DNAPTH (SEQ ID NO:3)	2164 A
	MAJORITY	CCCT CTT CGGCCGCGGGGGGCTAGGT GCCCGGACGT CAACGCCGGGTGAAGAGGCGTGCGGGGGGGGGG
•	ONAPTAD CNAPTR CNAPTR	
_	MAJORITY	G C G C G A T G G C C C A T G C C C C C C G C C C C C C C C C C C
	ONAPTAO CKAPTR CKAPTR	2304
	MAJORITY	TT C C C C C C C C C C C C C C C C C C
	ONAPTAD CNAPTR CNAPTR	23
	MAJORITY	CCAAAGAGGGGGGGGGGGKGGTGGGGGGTTTGGCCGAAGGAGGTGATGGAGGGGGGTGTATCCCCTGGCGT
	ONAPTAD CNAPTR CNAPTTH	. A A

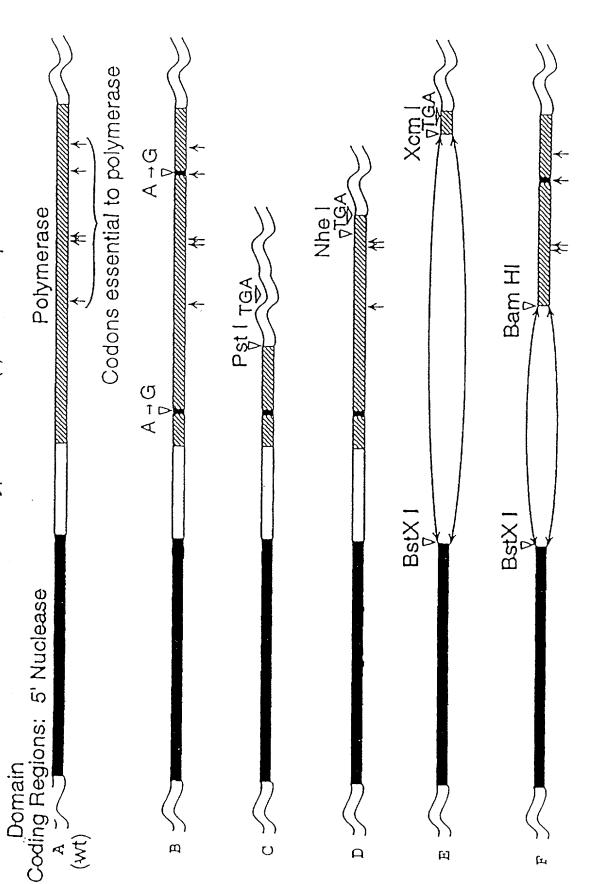
NO:7) GCCCCTGGAGGTGGAGGTGGGGATGGGGGAGGAGTGGCTCTCCCCCCAAGGAGTAG	
(SEQ ID NO:7)	OKAPTAD (SEQ ID NO:1) CKAPTR (SEQ ID NO:2) CKAPTR (SEQ ID NO:3)
MAJORITY	ONAPTAD (CNAPTR (

UORITY (SEQ ID NO:8)	MAJORITY (SEQ ID NO:8) MX A ML PL F E PKGRV LL V D G H H L A Y RT F F A L K G L T T S R G E P V D A V Y G F A K S L L K A L K E D G · D A V X V V F D A K	
TAO PRO (SEQ ID NO:4) TR PRO (SEQ ID NO:5) TR PRO (SEQ ID NO:6)	. Я G	70 7
MAJORITY	APSFRHEAYEAYKAGRAPTPEDFPROLALIKELVDLLGLXRLEVPGYEADDVLATLAKKAEKEGYEVRIL	
120 PRO 171 PRO 171 PRO	6G	139 138 140
MAJORITY	TADRDLYOLLSDRI AVLHPE GYLI TPAWLWEKY GLRPE OWV DYRAL X GDP SDNL P G V K G I G E KTAXKL L X	
720 PSO TH PSO TH PSO	K	209 208 210
МАЈОВПҮ	EWGSLENLLKNLDRVKP·XXREKI XAHMEDLXLSXXLSXVRTDLPLEVDFAXRREPDREGLRAFLERLEF	-
740 PRO TH PRO TH PRO	F O H O	278. 277 280
МАЛОВПУ	GSLLHEFGLLEXPKALEEAPWPPPEGAFVGFVLSRPEPMWAELLALAAARXGRVHRAXOPLXGLRDLKEV	
130 PRO TR. PRO TH PRO	SBBE.YKAA GAL.SFG.WE.LG.NFG. A.APA.AP	348 347 350

	-						1		
	4°° 4 420		488 487 490		\$\$8 \$\$7 \$60		628 627 630		698 697 700
POLLAKDLAVLALREGLDLXPGDDPMLLAYLLDPSNTTPEGVARRYGGEWTEDAGERALLSERLFXNLXX	S 6. P	RLEGEERLL WLYXEVEKPLSRVLAHME AT GVRL DVAY LOAL SLEVAEET RRLEEEVFRLAGHPFHLHSRD	K	OLERVLF DEL GLPAI GKTEKT GKRST SAAVLEAL REAHPI VEKI LOYRELTKL KNTYI DPL PXL VHPRT G	S D. 1	RL HT R F NOT A T A T G R L S S S D P N L O N I P V R T P L G O R I R R A F V A E E G W X L V A L D Y S O I E L R V L A H L S G D E N L		I RV F Q E G R D I H T Q T A S W M F G V P P E A V D P L M R R A A K T I N F G V L Y G M S A H R L S Q E L A I P Y E E A V A F I E R Y F O	
MAJORITY (SEQ ID NO:8)	(SEQ ID NO:4) (SEQ ID NO:5) (SEQ ID NO:6)								
MAJORITY	140 PR0 IR. PR0 IIH PR0	MAJORITY	140 P30 17. P30 11. P30	MAJORITY	140 P30 17, P30 171 P30	-MAJORITY	1AD PRO 1R, PRO 1R PRO	MAJORITY	120 PRO 171 PRO 171 PRO

4.00		
767 767 770		833 831 835
	F P R L X E MG A R ML L OV H D E L V L E A P K X R A E X V A A L A K E V ME G V Y P L A V P L E V E V G X G E D W L S A K E X	F 0. L D B A B W. 0 L C B A B A A A A A
TAD PRO (SEQ ID NO:4) TH PRO (SEQ ID NO:5) TH PRO (SEQ ID NO:6)	МАЈОВПУ	140 M30 IR M30 IH M30
	(SEQ ID NO:4) (SEQ ID NO:5) SEQ ID NO:6)	(SEQ ID NO:4) (SEQ ID NO:5) SEQ ID NO:6)

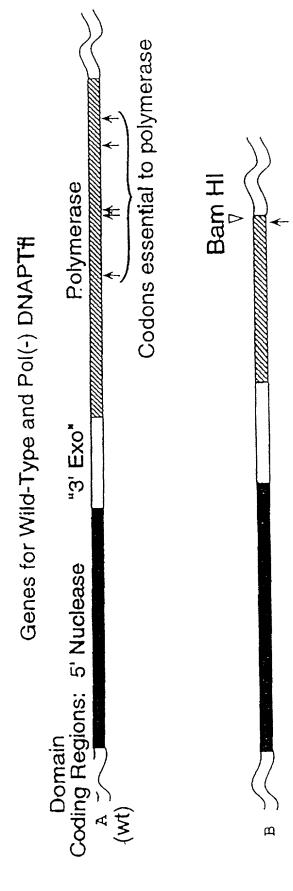
Genes for Wild-Type and Pol(-) DNAPTaq



14

FIGURE 5

Genes for Wild-Type and Pol(-) DNAPT#



Strand

FIGURE 6

A SHAPE AND A SHESS SUBSTRATE STRAIN

Cleavage a Sites Substrate Strain

CAGAATTCGAGCTCGC C

CAGAATTCGAGCTCGC C

CAGAATTCGAGCTCGC C

CAGAATTCGAGCTCGCGG

CAGAATTCGAGCTCGAGCGGG

Template Strand

Template Strand



FIGURE 8

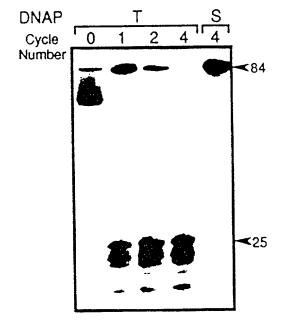
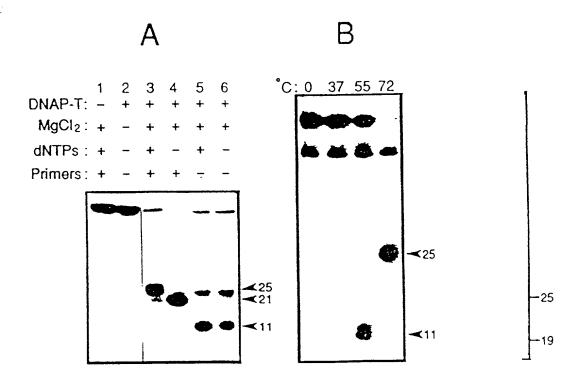


FIGURE 9



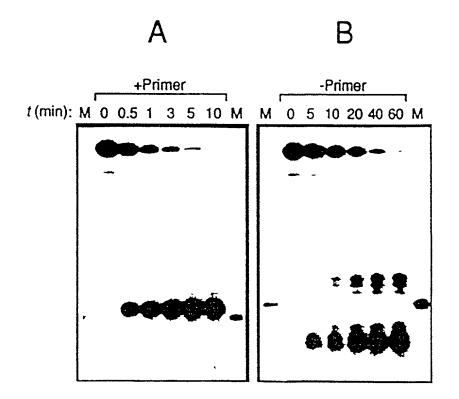


FIGURE 11

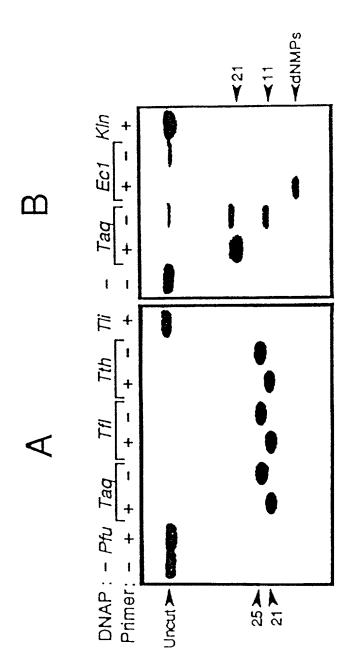
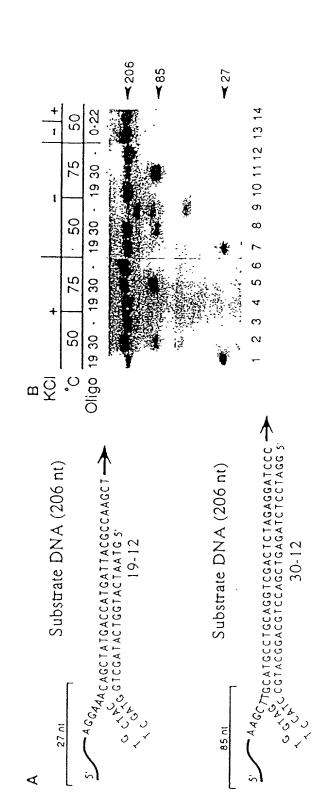
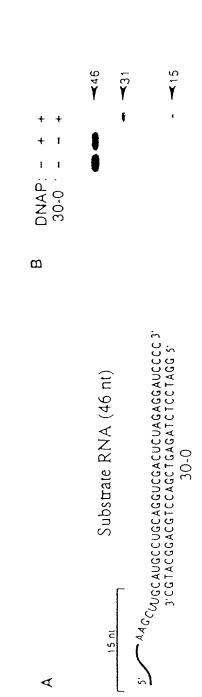
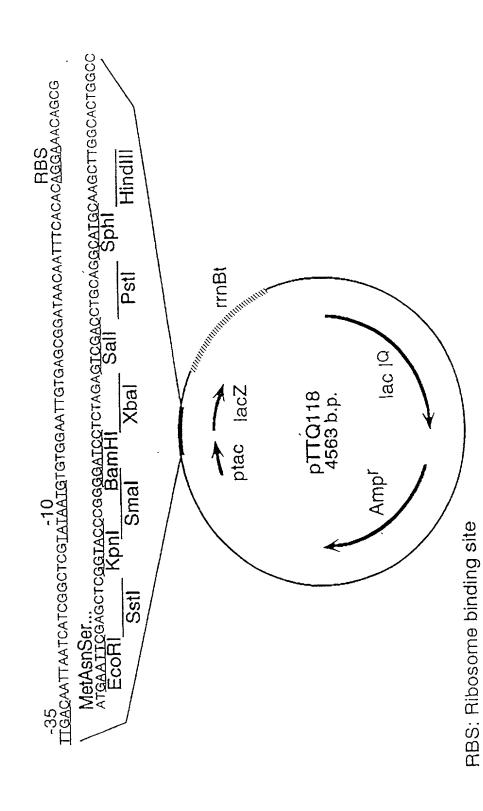


FIGURE 12





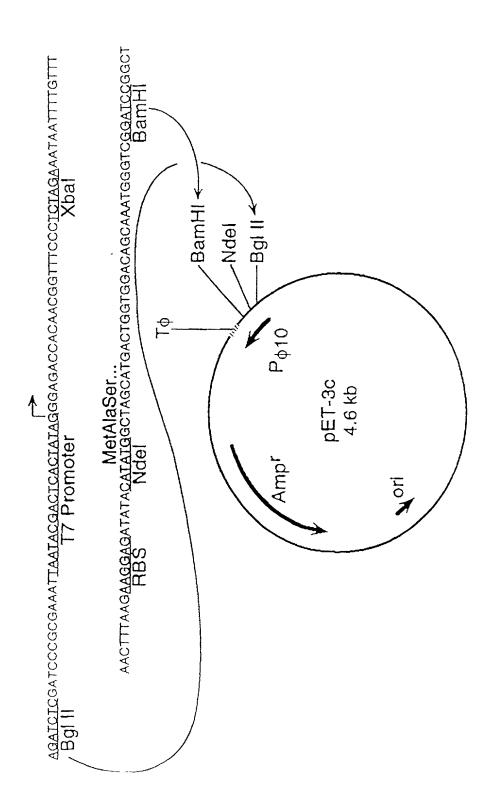


lacZ: Beta-galactosidase alpha fragment

ptac: Synthetic tac promoter

lac IQ: Lac repressor gene

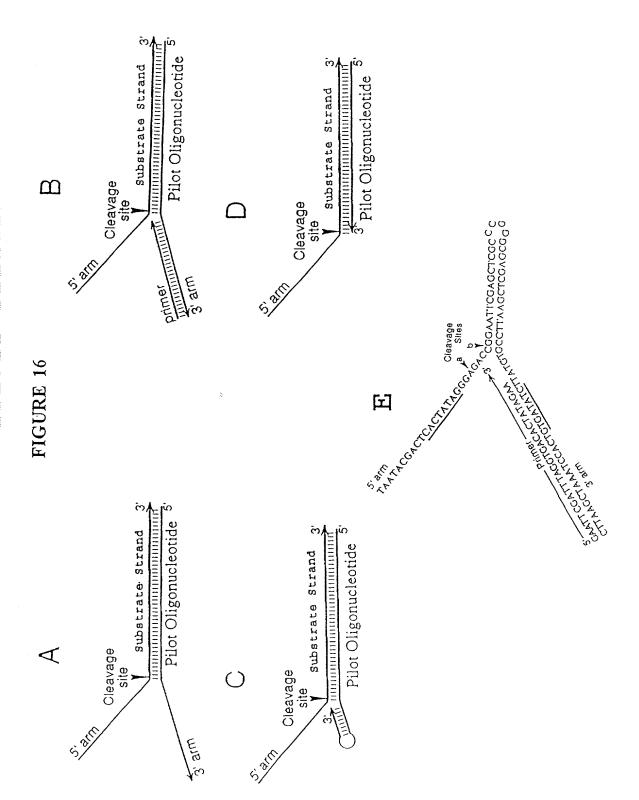
rrnBt: E. coli rrnB transcription terminator



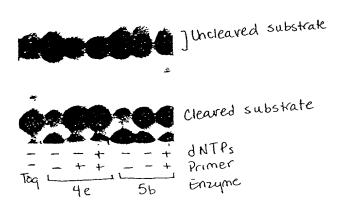
P_{\$0.10}: Bacteriophage T7 \$10 promoter T\$; T7 \$ Terminator

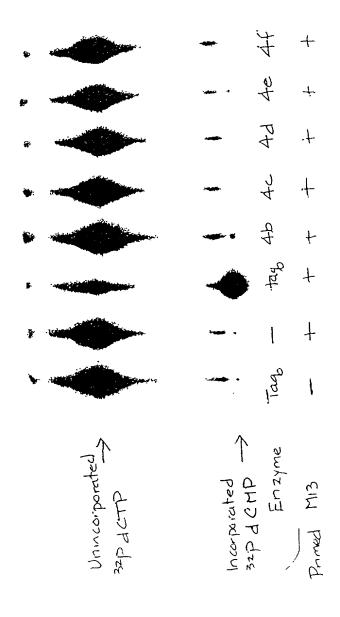
RBS: Ribosome binding site

25



1 2 3 4 5 6 7





A

Sites of Cleavage with a gap of 6 nt.

GATTTAGGIGACACTATAG

GATTTAGGIGACACTATAG

CTTAAGCTAAATCCACTGTGATATCTTATGTGCCTTA G

A

GATTTAGGIGACACTATAG

CTTAAGCTAAATCCACTGTGATATCTTATGTGCCTTA G

A

B

1 2 3 4 5 6 7 8

Carter of the second of the

84 nz - haurpin test molecule

(complete extension of primer)

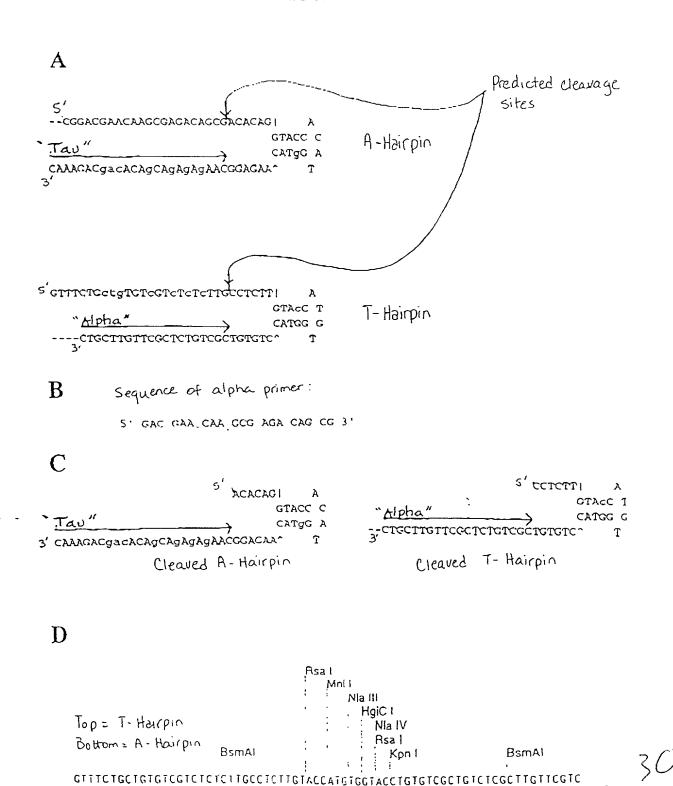
desired : >>
product
u nuc.

Multiple bonds

Caused by polymenzation

I some aberrant cleavage with 4b" because of residual polymerase activity.

29



CAAAGACGACACAGCAGAGAACGAGGAACATGGTACACCATGGACACAGCGACAGAGCGAACAAGCAGGC

Asp 718 Ava I Kpn I Xma I Sma I Bam HI XI GCSGTCCCAAAAGGGTCAGTGCTACATTTTGCTGCGGTCACTTAACATTATGCTGAGTGATATCCCGGCTTAAGCTCGAGCCATGGGCCCCTAGGAG CGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTGTAATACGACTCACTATAGGGCGAATTCGAGCTCGGTACCCGGGGATTCCT Ban II Sst I EcoR |

TAGAGICGACCIGCAGGCAIGCAAGCIIGAGIAJICIATAGIGICACCIAAAIAGCIIGGCGIAAICAIGGICAIAGCIGIIICCIGIGIGAAAIIGIIA A T C T C A G C T G G A C G T T C G A A C T C A T A A G A T A T C G C A A C C G C A T T A G T A C C A G T A T C G A C A A G G A C A C A C T T T A A C A A C --48 Reverse --Pst I BspM I Sph I Hind III — Pilot 30-0— Sall Acc | Hinc ||

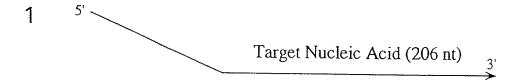
TCCGC TCACAATTCCACACATACGA

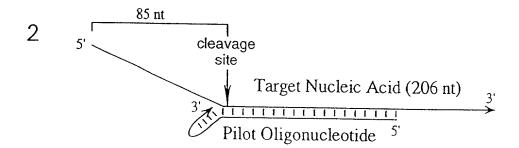
AGGCGAGTGTTAAGGTGTGTTGTATGCT

-48 kev 206

3/

FIGURE 22A





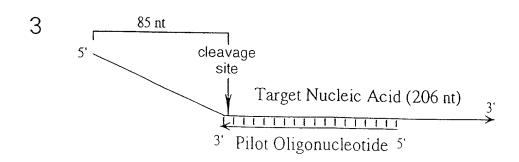
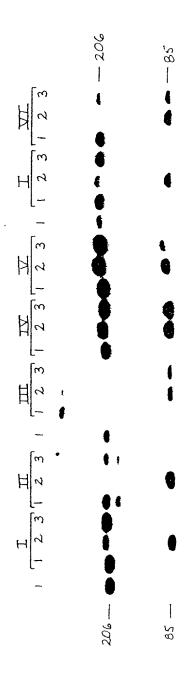
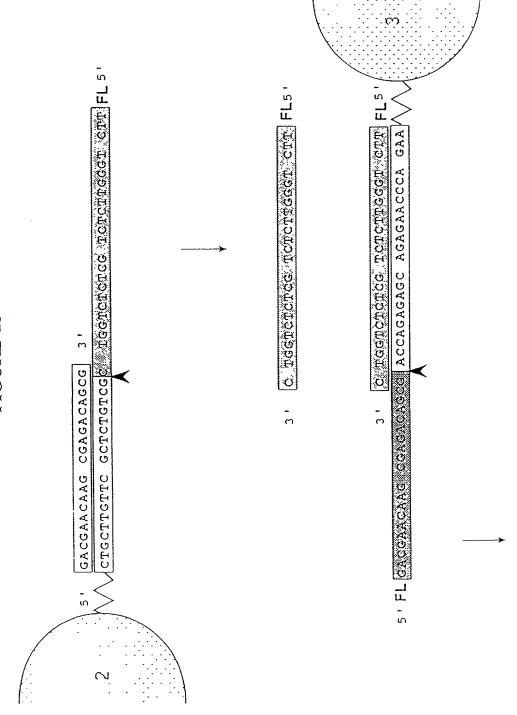
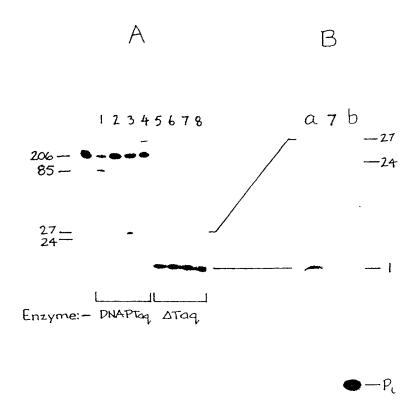
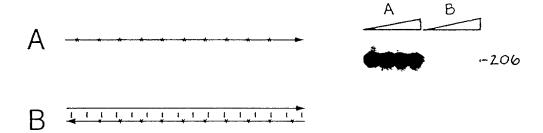


FIGURE 22B









$$* = {}^{32}P$$

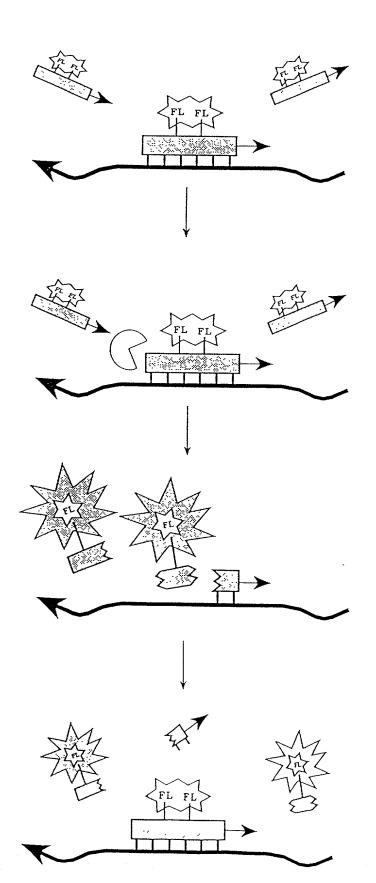
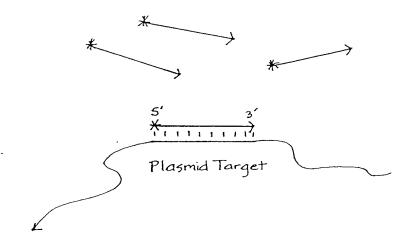
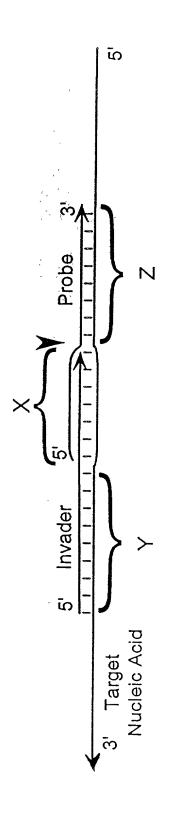


FIGURE 28A



* = 32 P 5' terminal phosphate

FIGURE 28B



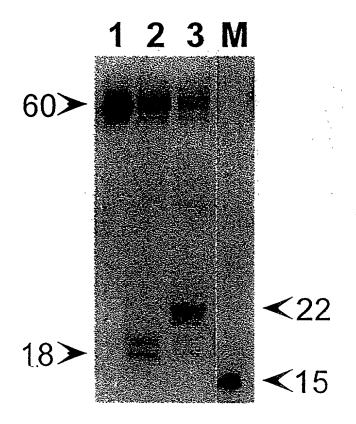
GCACAGAGCGAACA

3'CCCIICCAC

4'CCCIICCAC

5'CCCIICCAC

5'



s' g' Brobe s' GACGGGAAAGCGAAAGGAAAGGAAAGGAAAGGFluor. Target Nucleic Acid

ດັ Probe 3' GAAGGGAAAGG Fluor. - CTGCCCCTTTCGGCCGCTTGCACCGCTCTTTCCT Target Nucleic Acid 5' 3'5' GAAAGCCGGGAGAAGC

30g

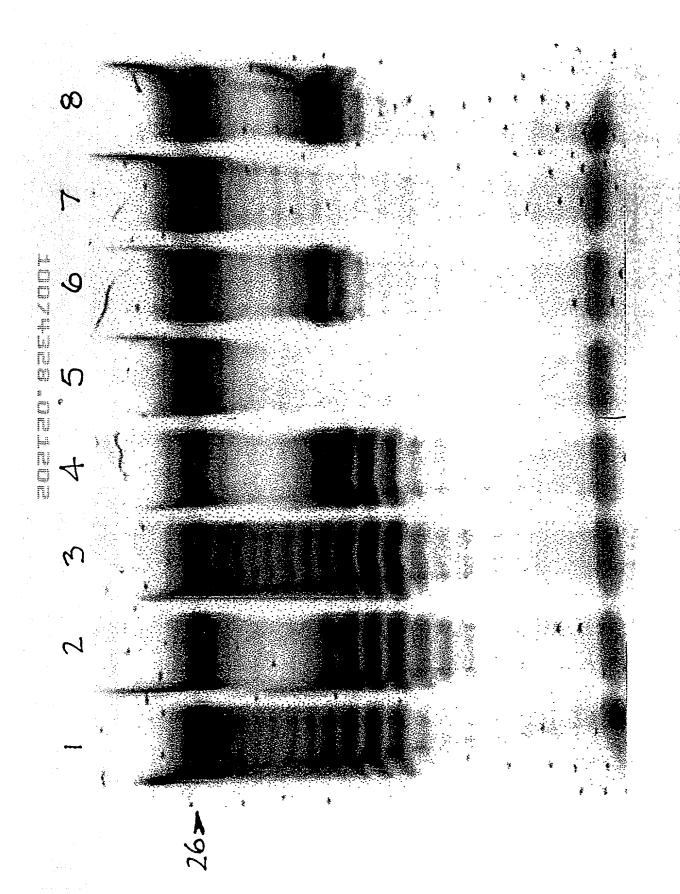
ည် - CTGCCCCTTTCGGCCGCTTGCACCGCTCTTTCCT

Target Nucleic Acid

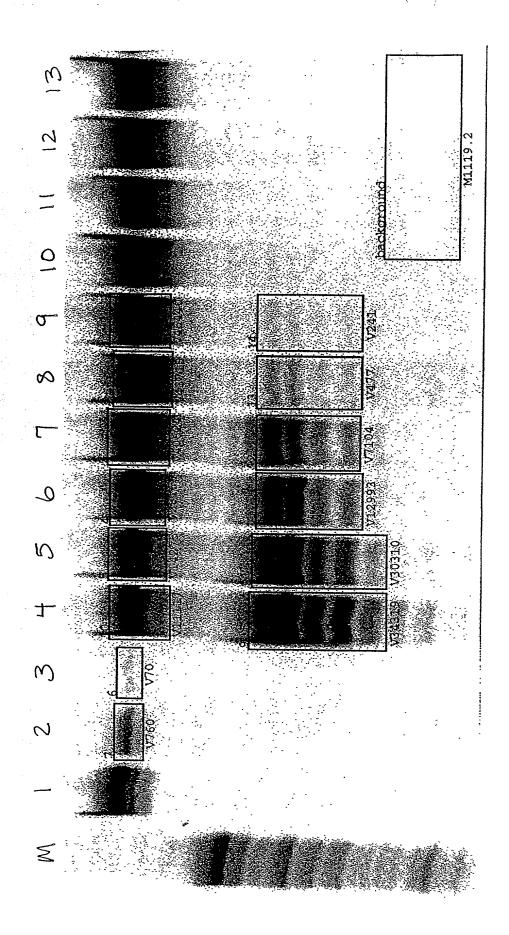
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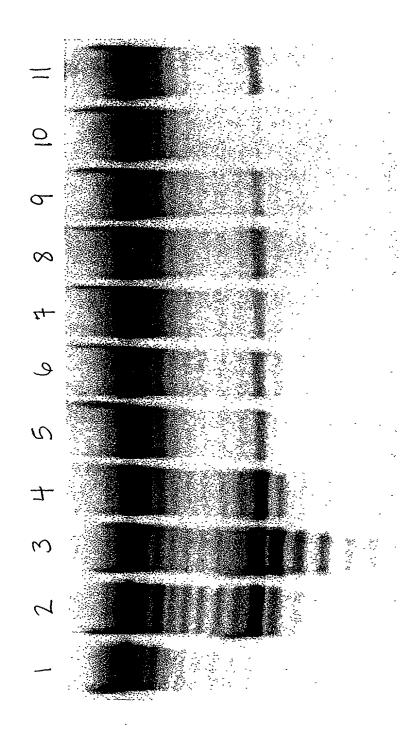
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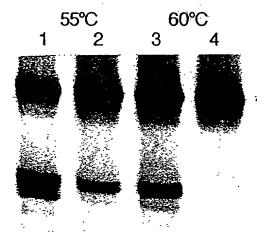
C

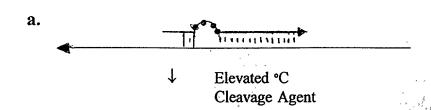


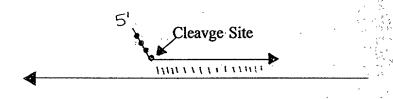
CC CALOBIA

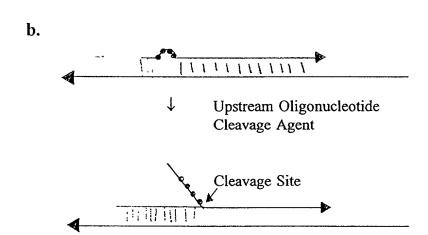










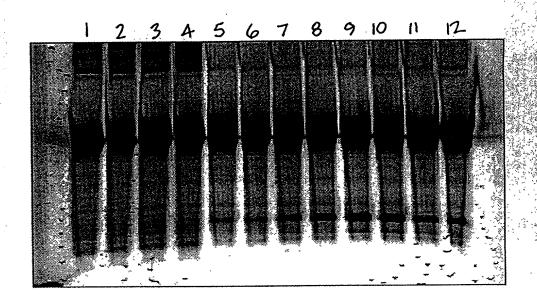


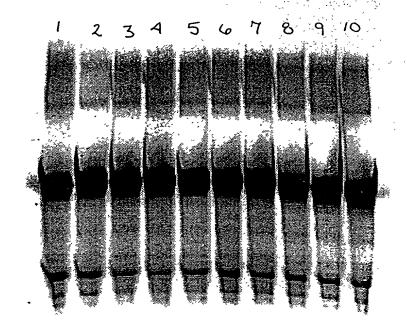
Invasive Cleavage Directing Oligo

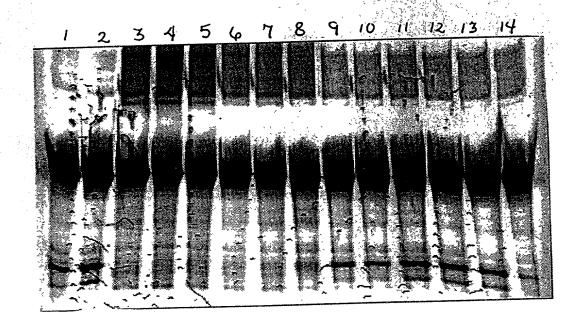
Sleavage site

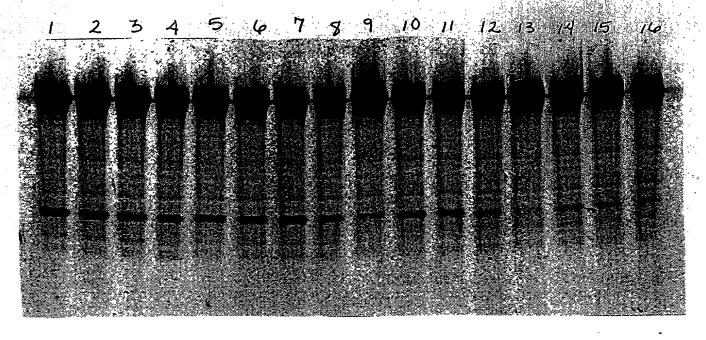
- Probe oligo 5'-ctgccctttcggcggcgcacgccgccctttcctttcgcttcc-.

Ť.









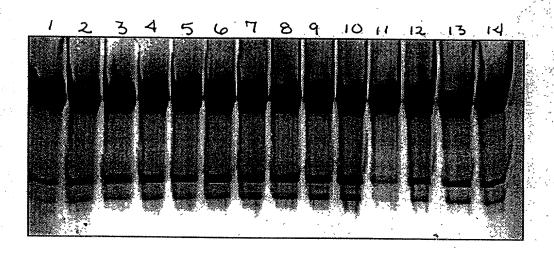
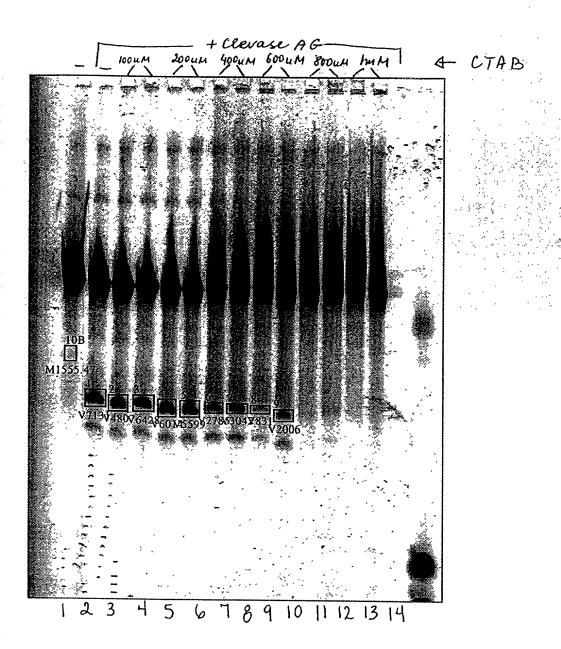


FIGURE 47



Sq

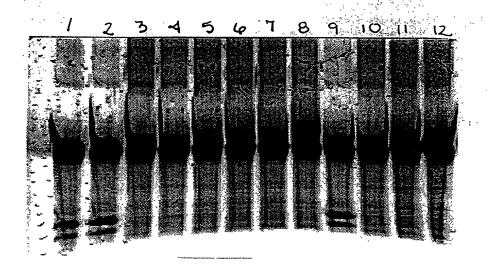
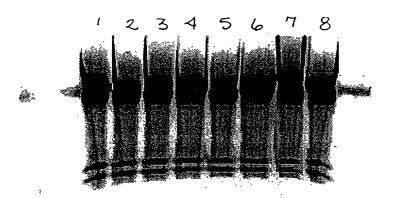
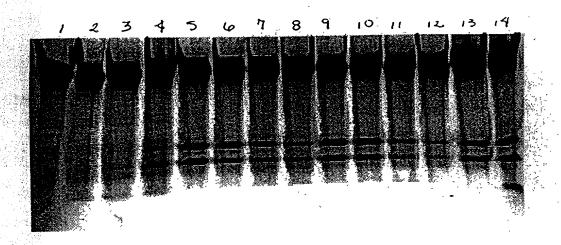
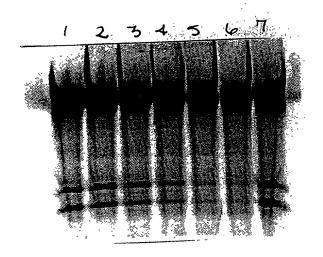
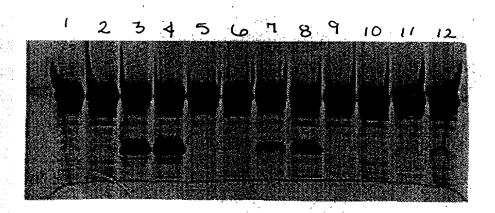


FIGURE 49









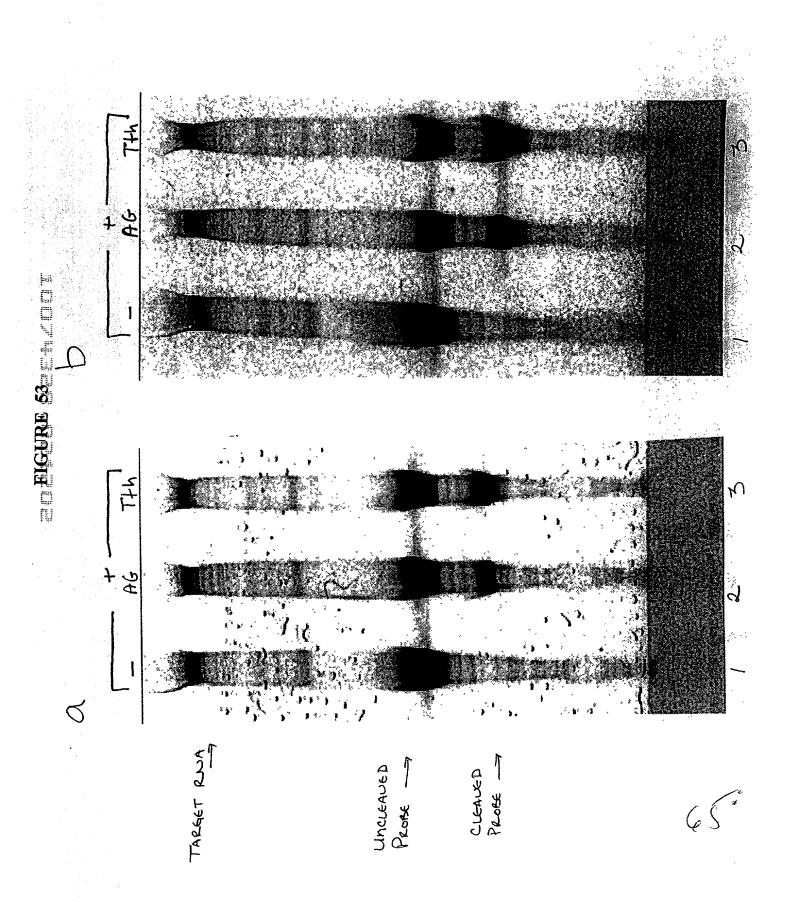


FIGURE 54

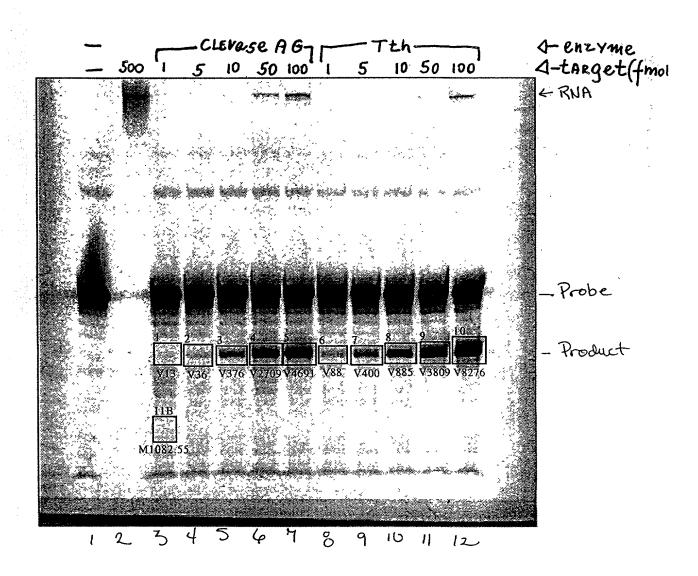
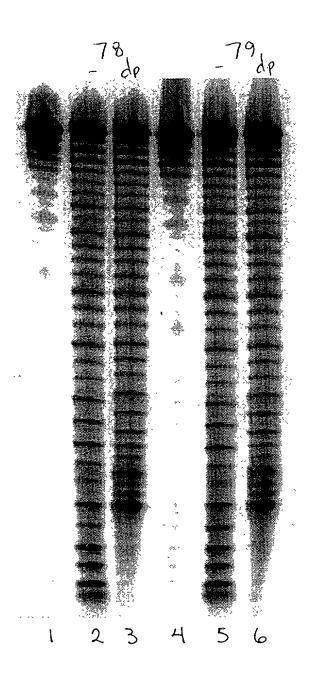
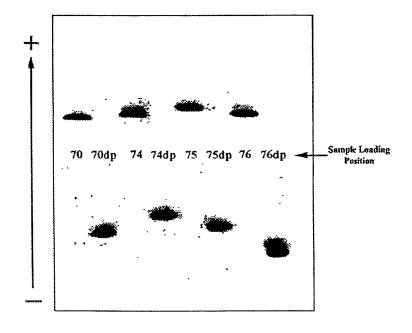
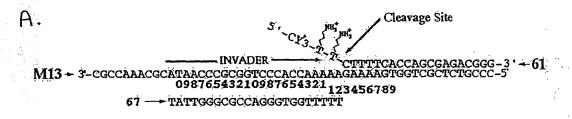


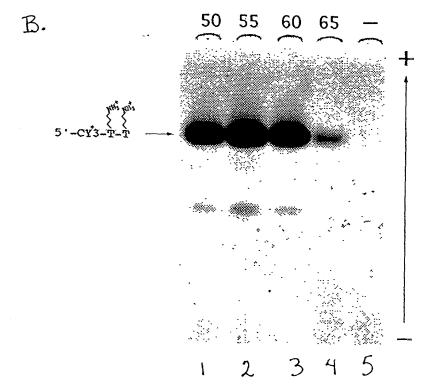
FIGURE 55

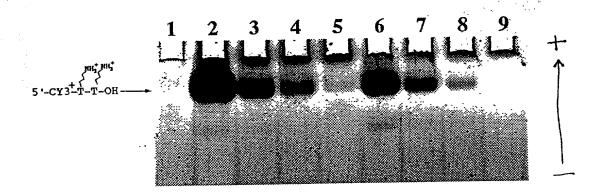


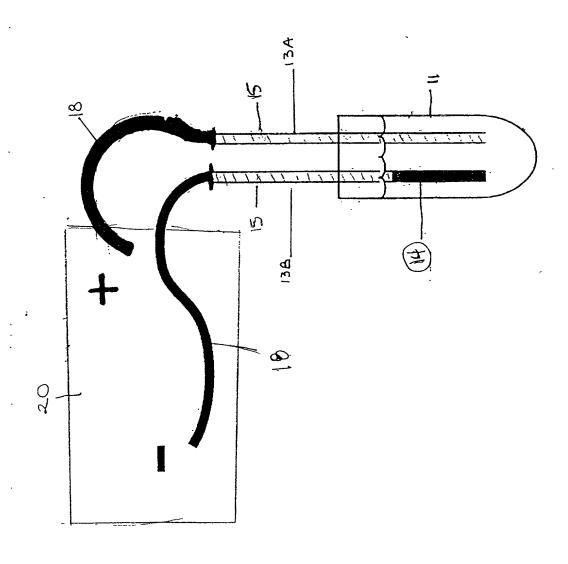
70 (C10 aminoT's) 74 (C6 amino T's)

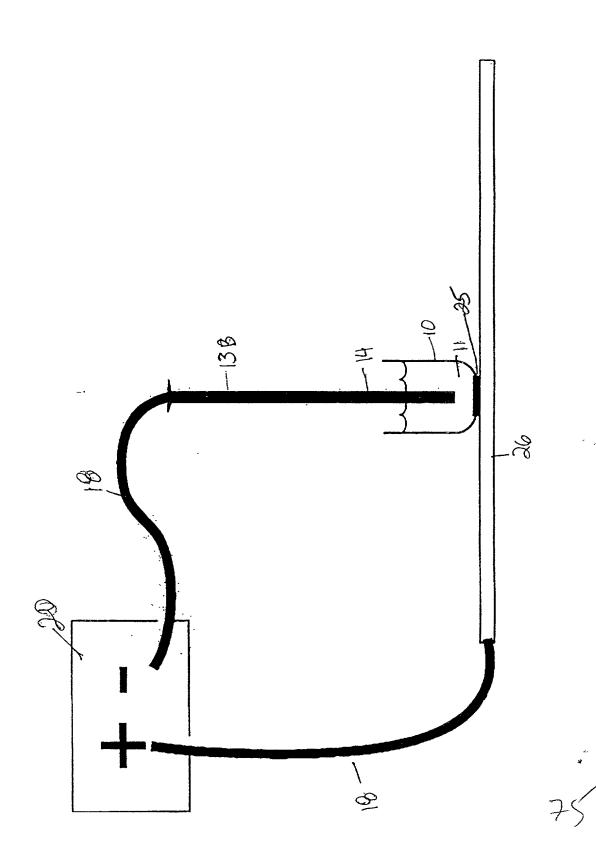


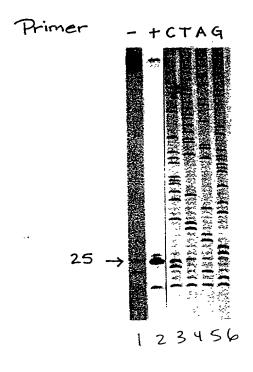












5' 3' 5' 3'	CGGCCGCTTGCACCGCTCTTTCCTTCCCTTCTTTCGCTTTCC	-	a
5' 3' 5' 3'		_	b
5' 3' 5' 3'	CGGCCGCTTGCACCGCTCTTTCCTTCCCTTCTTTCGCTTTCC CACCGCGCACCACCACCACCACCACCACCACCACCACCAC	31	
5' 3' 5' 3'	CGGCCGCTTGCACCGCTCTTTCCTTCCCTTCTTTCGCTTTCC CAGCGCGCTACCTCCCTTCTTCCCTTCC	3 '	

FED